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Specific Removal of Anti-acetylcholine Receptor Antibodies in Patients with Myasthenia Gravis

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■ In short-term therapy for myasthenia gravis caused by an antibody-mediated attack on the acetylcholine receptor (AChR) in skeletal muscle, a specific system for antibody removal, the use of tryptophan-bound immunoadsorbent and synthetic AChR peptide-bound immunoadsorbent, offers advantages over plasma exchange. These two types of immunoadsorption provided selective or semi-selective removal of pathogenic substances from the circulation without the use of plasma products, and minimized side-effects. A difficulty is that the former removed about 65% of the total IgG, and the latter removed only a fraction of the pathogenic antibodies. In neither case can a radical method of treatment for myasthenia gravis be expected. Hopefully, an adsorbent which has a well-balanced bioimmunological specific binding reaction and physicochemical adsorptive affinity will be developed in the future.

INTRODUCTION

Myasthenia gravis, a disease of neuromuscular transmission, is brought about by circulating antibodies raised against the nicotinic acetylcholine receptor (AChR) in skeletal muscle which cause the accelerated degradation of AChR, complement-mediated damage of the postsynaptic membrane and blockade of the ACh-binding site of AChR.[1] Based on this pathogenesis, the most important methods used currently in the treatment of myasthenia gravis include anti-cholinesterase agents, surgical thymectomy, immunosuppressive drugs (corticosteroids, azathioprine and cyclosporin), and short-term immunotherapies such as plasma purification and intravenous human globulin. Plasma purification is, in general, used to stabilize the condition in the severe myasthenic state or for the short-term treatment of patients undergoing thymectomy. It is sometimes used as an adjunct therapy in severely ill patients who are slow to respond to immunosuppressants. Repeated plasma purification may be useful as long-term therapy in patients who are either intolerant or unresponsive to conventional immunosuppressant therapies.[1] The methods of plasma purification have been devised using physical, chemical, or immunologic means with the hope of selective removal of anti-AChR antibodies.

TYPES OF PLASMA PURIFICATION

In the first step of plasma purification therapy, the patient's blood is directed into an extracorporeal circuit to be separated into plasma and red cells. The separation was previously performed by centrifuge, but in recent years, it has become common to use closed-loop continuous filtration through high molecular weight resin fibers. The blood is first heparinized and passed through a high molecular weight hollow fiber bundle where it is divided into plasma and

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cells. Since the blood is withdrawn and returned in a carefully interlocked closed circuit, loss of body fluid and treatment time are reduced, thus alleviating shock which could be caused by the treatment. The plasma purification therapy is divided into three broad categories, depending on how the plasma which is separated in the first step, is processed.

Plasma Exchange

All the plasma separated from the patient's blood is discarded, and normal human plasma or other fluid is fed through the circuit to replace the lost fluid, along with the red cells. All this is returned to the patient. Plasma exchange removes many substances ranging in size from low to high molecular weight. Due to the great reduction of protein components which occurs during this process, a large amount of replacement plasma is required. This increases the risk that the patient may contract hepatitis or HIV virus infection from the replacement plasma.

Double Filtration Plasmapheresis

The filter which separates the blood into blood cells and plasma is called the first filter. The ultrafiltration filter which fractionates the plasma components according to their molecular weight is called the second filter. It fractionates protein components with molecular weights in the range of 20,000 or 30,000–200,000 or 300,000 Da. Usually second filters have a cut-off value of about 20,000 or 30,000 Da, to block gamma-globulin fractions while letting albumin fractions pass through the circuit. The volume of plasma to be processed per session is usually 50 mL/kg body weight. The procedure is often conducted on a daily basis or 3–5 times every other day during the course of treatment. The amount of gamma-globulin removed is about 10–20% of the total volume of plasma processed, although it may vary with the influx pressure of the second filter and the duration of each treatment. This means that when 3000 mL of plasma is processed three successive times, 80–90% of the IgG can be removed from the plasma. The plasma albumin level also drops somewhat, so albumin replacement is also often performed to compensate for this reduction. This procedure may increase the risk of infectious diseases, because essential plasma constituents are also removed by the treatment.

Immunoadsorption

A column containing an adsorbent or ligand-linked carrier is installed, instead of the second filter used in the double filtration plasmapheresis, to selectively or semi-selectively remove target antibodies from the plasma. The following two types of adsorbents are currently in use for the treatment of myasthenia gravis.

TR350®. This is an adsorbent consisting of tryptophan-linked polyvinyl alcohol resin (Fig. 1).[2] It absorbs anti-AChR antibodies through hydrophobic interaction. The results of an *in vitro* experiment using TR350® revealed that the removal rate of anti-AChR antibodies was about 70%.[3] This adsorbent is used also for the treatment of Guillain–Barré syndrome. PH350®, an adsorbent produced by linking phenylalanine to the same carrier as TR350®, is not indicated for patients with myasthenia gravis, because the removal rate of anti-AChR antibodies is as low as 35%.

MG50®. Molecular information about the primary structure of the AChR pre-

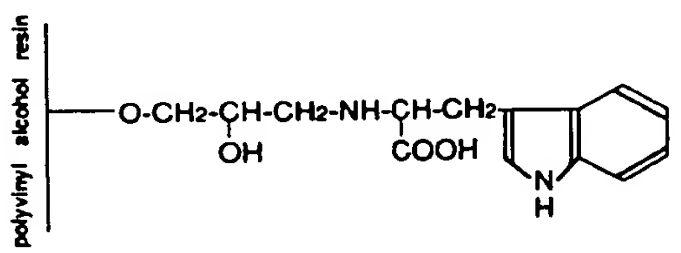

Figure 1. Polyvinyl alcohol resin linked with tryptophan through epichlorohydrin (TR350®). The capacity of the column is 350 mL.

cursor[4] and the predicted transmembrane passage[5] has been obtained, current work based on these data has contributed to an understanding of the possible localization of the myasthenic domain on AChR at a molecular level. Advances in molecular and biological studies of AChR and in techniques for determining anti-AChR antibody specificity now permit us to define the antigenic structure of AChR and the heterogeneic complexity of antibodies.[6,7] Selective removal of antibodies from individual patients by plasma perfusion with a sorbent that is specified as a pathogenic antigen (B cell epitope) is thus one of the modern strategies of immunotherapy for myasthenia gravis. Prior to the designation of immunoadsorption to remove specific antibodies, however, it is essential to define the myasthenogenic sites on the amino acid sequences of AChR which are involved in the production of antibodies. We reported that the segment of residues 183–200 of AChR α-subunit is involved in the formation of the ACh-binding site, and the peptide synthesized referring to its Torpedo sequence is immunogenic for the production of the animal model of immunopharmacologic blockade of the ACh-binding site.[8] Our previous study also demonstrated that this synthetic peptide (Torpedo sequence) can be antigenic for the detection of antibody in human myasthenic serum,[8] and thus suggested that the peptide may be a useful adjunct to the antigen-specific therapy for myasthenia gravis. With the idea drawn from these results, the immunoadsorbent MG50® (Fig. 2) has been developed by synthesizing the peptide (Torpedo α183–200) and linking it to an insoluble carrier or porous cellulose particles.

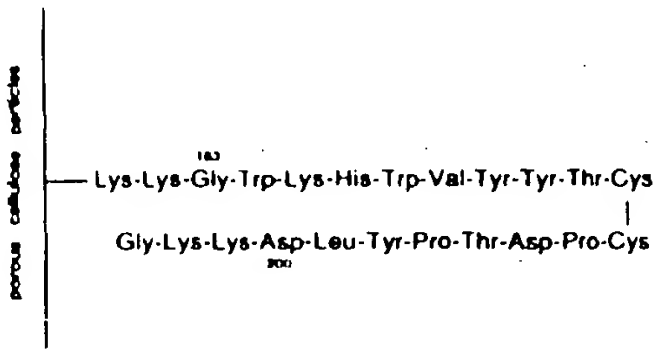

Figure 2. Adsorbent linked with synthetic peptide of acetylcholine receptor (MG50®). Cellulose particles linked with peptide corresponding to the residues 183–200 of Torpedo acetylcholine receptor α-subunit by the hydrophilic amino acid at each end. The capacity of the column is 50 mL.

Protein A. Sepharose–protein A (Prosorba) was intended to adsorb IgG subclass substances. According to results from an *in vitro* experiment, this adsorbent removed 50–60% of subclasses IgG 1 and 2, or anti-AChR antibodies.[9] However, it also has a significant incidence of side-effects and, thus, there has been no report of the clinical application of this adsorbent for the treatment of myasthenia gravis.

THERAPEUTIC EFFICACY

Plasma Exchange and Double Filtration Plasmapheresis

The discovery that myasthenia gravis is caused by anti-AChR antibodies in the blood has naturally led to the idea of trying to remove them in order to treat the disease. As early as 2 years after the confirmation of pathogenesis, Pinching and Peters used this concept in clinical practice.[10] In their study, they performed plasma exchange on two myasthenic patients, finding that the removal of anti-AChR antibodies was effective, as predicted by the theory. They also pointed out that the therapeutic effect was short-lived, suggesting the necessity of concurrent use of immunosuppressive drugs. Follow-up studies performed by others also supported the idea that it is necessary to use steroids or azathioprine in combination with plasma exchange to prolong the therapeutic effect.[11,12] Behan and co-workers reported that 21 patients with myasthenia gravis were treated by plasma exchange in combination with steroids or azathioprine with good results in 15 patients, 60% of whom showed remissions lasting up to 9

months; anti-AChR antibody titers remained low in parallel with clinical improvement.[11] Fornadi and co-workers, who had observed 160 patients with myasthenia gravis for 10 years, reported that plasma exchange had a good therapeutic effect in 63% of patients during the first series of treatments and also provided a good effect in additional treatment series in 30% of patients.[13] Also reported is that when severe myasthenic patients underwent plasma exchange before thymectomy, they were able to leave the ICU or be taken off the respirator earlier than those who did not undergo the plasma exchange.[14] The study, based on electrophysiological estimates, showed that improvement began 1–7 days after the start of plasma exchange.[15,16] Stricker and co-workers argued that plasma exchange is more effective for patients in myasthenic crisis than is the large-volume immunoglobulin therapy which has drawn attention in recent years.[17] As suspected by the difference in the circuit employed for plasma perfusion, double filtration plasmapheresis is more selective in IgG antibody removal and safer than plasma exchange; its use has become widespread in Japan and achieves the same therapeutic effect as plasma exchange.

Immunoadsorption

TR350®. Shibuya and co-workers,[18] in a clinical study, performed a series of five immunoadsorption treatments using TR350® for 20 patients with severe generalized myasthenia gravis in crisis. They were concurrently given prednisolone and azathioprine. As a result, an excellent therapeutic effect was seen in 11 patients. In particular, the treatment was effective for those without thymoma. Muscle strength scores improved an average of 39% immediately after the end of treatment and the improvement lasted at least 1 month. On average, anti-AChR antibody titers decreased to 36% of the pretreatment value immediately after the end of treatment, and thereafter increased gradually to the pretreatment level 1 month later. Serum albumin decreased by only 6%.

Grob and co-workers[19] reported that among 14 myasthenic patients who were treated with immunoadsorption therapy using TR350®, 12 patients (86%) showed an excellent or good result. Improvement began about 42 h after the start of treatment and reached a peak at day 4 after the fourth session. Muscle strength scores increased 2.5 times and returned to the pretreatment level in 2 months. Serum proteins were reduced 39 g by one session, but less than the 175 g reduction after the plasma exchange control procedure. Anti-AChR antibodies and IgG decreased to 23 and 35% of the pretreatment level, respectively. Albumin remained at 77% of the pretreatment value; there was thus no need for supplementation of albumin. The level of serum proteins returned to the pretreatment values within 1–3 weeks after the end of treatment. It is safe to say that immunoadsorption therapy using TR350® is superior to plasma exchange or double filtration plasmapheresis. The problem is that it removes about 65% of the total IgGs. There seems scope for the selectivity of this procedure to be improved.

MG50®. A pilot clinical study of immunoadsorption therapy using MG50® as an adsorbent was performed on two myasthenic patients.[20] Plasma perfusion using this adsorbent resulted in a progressive decline of the anti-Torpedo α183–200 peptide antibody in cases 1 and 2 (Fig. 3). This treatment specifically reduced the anti-peptide antibody by the end of the third treatment, as confirmed by its marked reduction (26 and 42% of the immediate pretreatment values, respectively) compared to the alteration of total IgG (82 and 71% of the immediate pretreatment values, respectively) (Fig. 3). The anti-native AChR blocking antibody, directed against the ACh-binding site of AChR, was also reduced significantly in both cases (55 and 54% of the immediate pretreatment values, respectively; Fig. 3). Reduction

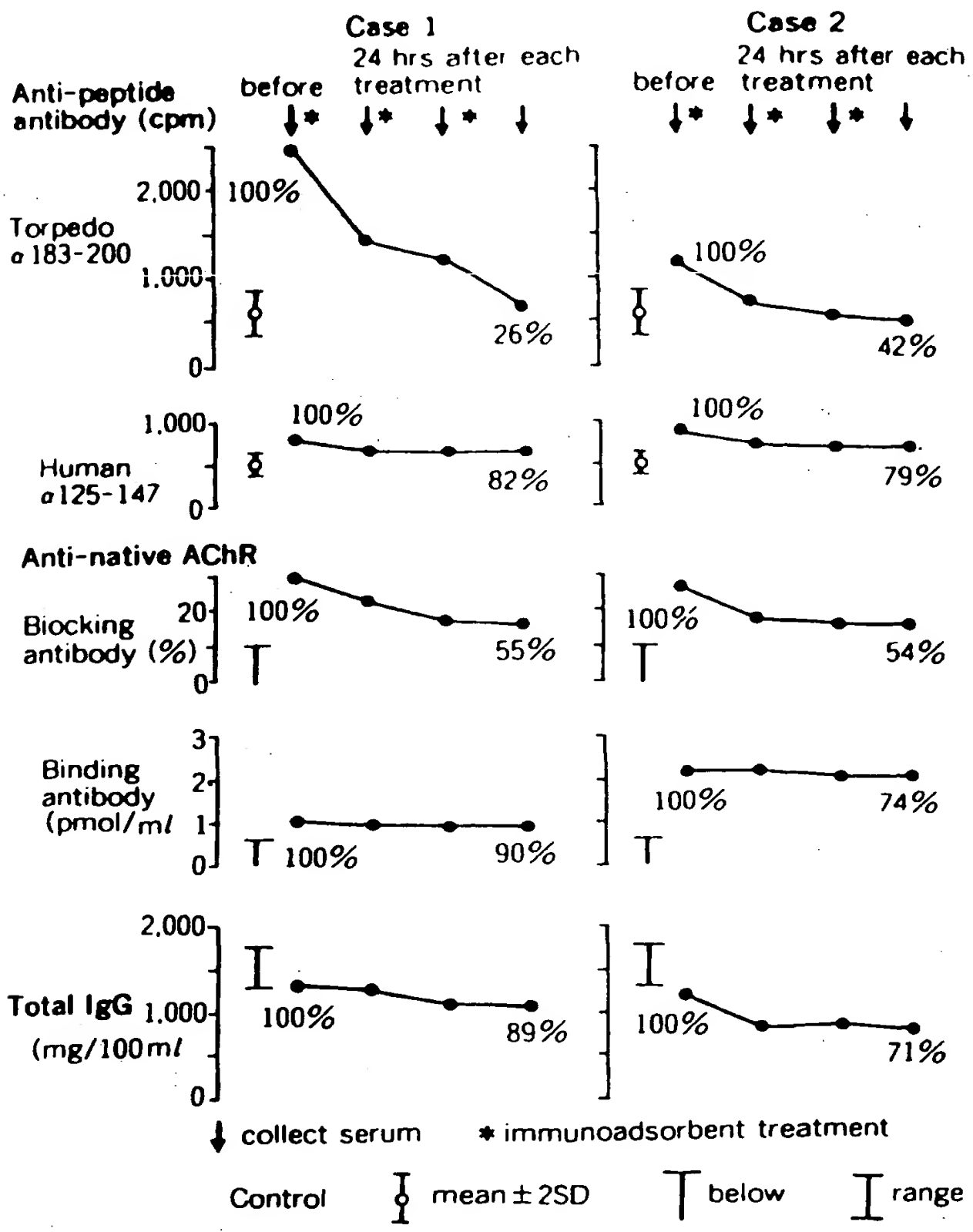

Figure 3. Effect of immunoadsorption therapy by MG50® (Fig. 2) in two patients with myasthenia gravis. Treatments were performed by 40 mg peptide/2000 mL plasma in case 1, and 30 mg peptide/2000 mL plasma in case 2. Changes of anti-Torpedo α183–200 antibody by the glycine-containing adsorbent (without peptide) (control) *in vitro*: from 2465 to 2358 cpm in case 1; from 1122 to 1075 cpm in case 2.

in the antibody to unrelated peptide (human α125–147) and the anti-native AChR binding antibody, directed against sites other than the ACh-binding site and implicated in the accelerated AChR degradation, was within the range of reduction in total IgG (Fig. 3). Seven and 28 days after the series of immunoadsorption treatments, the anti-peptide (Torpedo α183–200) antibody was 538

and 566 cpm in case 1, and 658 and 410 cpm in case 2. As far as short-term observation was concerned, they were reduced significantly and fell to within the normal range (Fig. 3). Electrophysiological indices responded to the repeated immunoadsorption treatment as manifested by an increase in amplitude of single evoked muscle action potentials and a reduced decrement in successively evoked muscle action potentials at 2 Hz in both cases (Fig. 4). Using the same procedure,

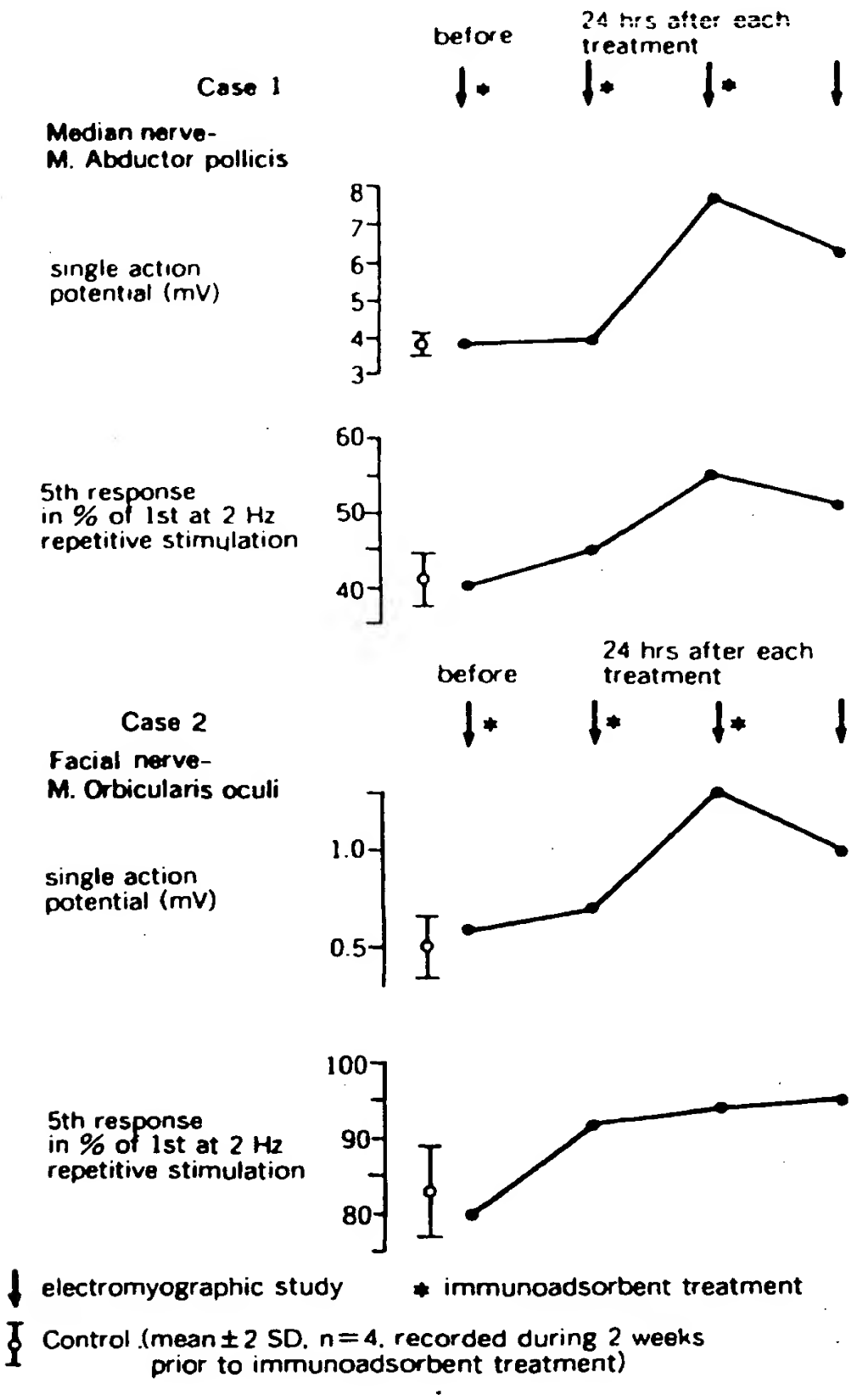

Figure 4. Changes in electrophysiological indices of neuromuscular transmission, aligned with respect to days of immunoadsorption therapy by MG50® (referred to Fig. 3) in two patients with myasthenia gravis.

an additional clinical study was performed on 22 patients with myasthenia gravis. The electrophysiological examination performed along with a series of treatments showed that an improvement was seen in 57% of the patients and lasted about 7–10 days. The antinative AChR blocking antibody titers dropped in 69% of the patients treated. The total IgG fell only to about 85% of the pretreatment level. It seems that the synthetic peptide-bound adsorbent is superior to the tryptophan-bound adsorbent. The conformational modification of the synthetic peptide by linking artificial sequences of amino acids will potentiate the capability of the adsorbent to specifically remove the antibody.[21,22] However, this synthetic peptide-bound adsorbent poses the problem that it is so selective that only the anti-AChR blocking antibodies, directed against the ACh-binding site of AChR, one set among the various anti-AChR antibodies implicated in the pathogenesis of myasthenia gravis, are removed.[8]

MODE OF ACTION

It is generally assumed that plasma purification therapy helps treat myasthenia gravis through any or a combination of the following actions: (1) alleviation of the blockade of AChR function due to a reduced anti-AChR antibody titer in the blood, (2) normalization of the AChR degradation rate and increase in the concentration of neogenic AChR on the synaptic membrane surface, (3) decrease in the production of anti-AChR antibodies by B-cells, secondary to a reduced level of interleukin 2, (4) activation of complement C3 through the action of the filtration membrane,[23] and (5) suppressed production of antibodies due to the activation of suppressor-inducer T-cells.[24] Undesirable effects of this plasma purification may include: inability to neutralize the anti-AChR antibodies brought about by the removal of anti-idiotype antibodies, and acceleration of the production of antibodies by the activation of helper T-cells following the activation of complement C5.[24]

SIDE-EFFECTS

Thrombosis due to coagulation is one of the inevitable side-effects of extracorporeal blood circulation. The procedure reduces anti-thrombin III, causing an increase in coagulation factors, thus causing thrombosis rather than bleeding. Except for thrombosis, the side effects produced by the procedure are few; such severe side-effects that treatment must be suspended rarely occur when a skilful physician carries out the treatment.[25]

PERSPECTIVES ON FUTURE DEVELOPMENT

The purpose of plasma purification therapies, including plasma exchange, double filtration plasmapheresis and immunoadsorption, is to remove pathogenic autoantibodies already present in the circulating blood. These therapies are not a radical modality of treatment for myasthenia gravis. There are still a number of unknowns concerning the production of antibodies and the pathophysiology of myasthenia gravis. If, in future, any factor is found which enforces the functions of the specific T and B cells involved in accelerating the production of antibodies, the removal of such a factor will not only lead to the establishment of a true radical therapy but will also lead to so-called immunological modulation. For this reason, we should make further improvements in the currently used adsorbents, so as to be able to selectively remove specific antibodies and specific cytokines, instead of removing all antibodies. The clinician must be prudent when using high-molecular-weight specific antigens as adsorbents, because the antigen may leave the carrier and enter into the body circulation, thus causing the risk of another immunoreaction. When it

comes to low-molecular-weight adsorbents, enhancing their adsorptive specificity is one of the most important problems to be solved. It is hoped that an adsorbent which has a well balanced bioimmunological specific binding reaction and physicochemical adsorptive affinity will be developed.

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